Morphological and Molecular Evidence for Synonymy of Cinclidotus confertus Lüth with C. riparius (Host ex Brid.) Arn

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Morphological and molecular evidence for synonymy of *Cinclidotus confertus* Lüth with *C. riparius* (Host ex Brid.) Arn.

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ABSTRACT
Cinclidotus confertus Lüth was described in 2002 from Greece and has to date only been recorded from the type locality. The taxon was supposed to differ from the widespread *C. riparius* (Host ex Brid.) Arn. in the red and papillose peristome teeth, as opposed to yellow and smooth peristome teeth of *C. riparius*. However, we found that the peristome of *C. riparius* was inconsistently described in the bryological literature, with some authors admitting reddish and papillose peristome teeth as well. To clarify the peristome characteristics of *C. riparius* and the taxonomic identity of *C. confertus* we studied both taxa morphologically and molecularly. Sporophyte characteristics of *C. riparius* are variable, but most specimens have red and papillose peristome teeth, and no other morphological difference could be found between plants assigned to *C. confertus* and *C. riparius*. The many erroneous or incomplete descriptions of the peristome characteristics of *C. riparius* may be related to the fact that in central Europe sporophytes are rarely produced and that the fragile peristome easily erodes. Furthermore, the phylogenetic analysis of chloroplast *rps4* and nuclear ITS2 loci failed to segregate specimens assigned to *C. riparius* from those assigned to *C. confertus*, including material from the type locality and further newly discovered localities of the latter taxon. Consequently, we propose the synonymy of both taxa.

KEY WORDS
Aquatic mosses,
ITS2,
Pottiaceae,
rps4,
Europe,
Greece,
new synonym.

RÉSUMÉ

Synonymie de Cinclidotus confertus *Lüth avec* C. riparius *(Host ex Brid.) Arn. sur la base d'analyses morphologiques et de biologie moléculaire.*

Cinclidotus confertus Lüth a été décrit en 2002 de Grèce et n'est pas connu en dehors de sa localité type. Le taxon est supposé différer de *C. riparius* (Host ex Brid.) Arn., à large distribution, par les dents du péristome rouges et papilleuse, tandis que chez *C. riparius* elles sont jaunes et lisses. Cependant, nous avons trouvé que le péristome de *C. riparius* était décrit de façon inconsistante dans la littérature bryologique, où quelques auteurs admettent des dents du péristome rougeâtre et papilleuses. Pour clarifier les caractéristiques du péristome de *C. riparius* et l'identité taxonomique de *C. confertus* nous avons étudié leur morphologie et leur biologie moléculaire. Les caractéristiques du sporophyte de *C. riparius* sont variables, mais de nombreux spécimens ont des dents du péristome rouges et papilleuses, et aucune autre différence morphologique ne peut être trouvée entre les plantes assignées à *C. confertus* et *C. riparius*. Les nombreuses erreurs et les descriptions incomplètes des caractères du péristome de *C. riparius* peuvent être dues au fait qu'en Europe centrale les sporophytes sont rarement produits et que le péristome fragile s'érode facilement. En outre, l'analyse phylogénétique des loci chloroplastique *rps4* et nucléaire ITS2 ne permet pas de séparer les spécimens attribués à *C. riparius* de ceux assignés à *C. confertus*, en prenant en compte du matériel provenant de la localité type et de nouvelles récoltes de ce dernier. En conséquence nous proposons la synonymie des deux taxons.

MOTS CLÉS
Mousses aquatiques,
ITS2,
Pottiaceae,
rps4,
Grèce,
Europe,
synonyme nouveau.

INTRODUCTION

Cinclidotus confertus Lüth was described from a single locality in northern Greece (Lüth 2002). Since then, the species has not been reported from any other site or country (see e.g. Hodgetts 2015). In the protologue of *C. confertus* it was compared to the widespread *C. riparius* (Host ex Brid.) Arn., which is similar in gametophytic characteristics including habit, leaf shape and the dioicous sexual condition. The separation of *C. confertus* from *C. riparius* was based on sporophyte characteristics (Lüth 2002). For *C. riparius*, the peristome has been reported to be yellow and (almost) smooth in the standard bryological literature (Limpricht 1890; Amann & Meylan 1912; Mönkemeyer 1927; Burck 1947; Pedrotti 2001; Smith 2004), whereas in *C. confertus* the teeth are red and papillose (Lüth 2002). However, in a recent revision of *Cinclidotus* in Turkey (Erdağ & Kürschner 2011), the colour of the peristome in *C. riparius* is described as variably yellowish to orange and red. Although Erdağ & Kürschner (2011) studied and recognized *C. confertus*, they assigned specimens with red peristome to *C. riparius*. The peristome surface of *Cinclidotus riparius* is described as nearly smooth to slightly papillose by these authors, in accordance with Frahm & Frey (1992), who describe it as slightly papillose. Similarly, Ederra (2006) characterises the peristome of *C. riparius* as being yellowish orange and finely papillose. These inconsistencies in descriptions lead to doubts about the taxonomic value of *C. confertus*.

Cinclidotus riparius was described as *Gymnostomum riparium* Host ex Brid. by Host (1797), but with respect to the starting point for the mosses, the description was first validated by Bridel (1801), who only mentioned the name of the species. Host (1797), with respect to the generic assignment, regarded the species as eperistomate, probably because of the fragile peristome, which may have fallen off. Mohr (1806) placed *G. riparium* in *Trichostomum* Hedw. and in the same year, Bridel (1806) validly published another similar species in this genus, *Trichostomum nigricans* Brid. Bridel (1806) did not describe the peristome, probably because the peristome in the type specimen was already eroded and most likely at the time he described the species he was not aware of *T. riparium* because he did not mention the similarity of that species. Subsequently however, *T. nigricans* was consistently treated as a synonym of *T. riparium* throughout the bryological literature by various authors including Bridel himself (Weber & Mohr 1807; Schkuhr 1810; Schwaegrichen 1811; Bridel 1819; Bruch *et al.* 1842; Limpricht 1890; Nebel & Philippi 2000; Smith 2004; Ederra 2006; Frey *et al.* 2006; Erdağ & Kürschner 2011). The first description of the colour of the peristome of *C. riparius* (as *T. riparium*) was provided by Schkuhr (1810), whose illustration plate shows a distinctly red peristome. However, it is unclear where the underlying specimen originated from. In contrast, Hedwig (Schwaegrichen 1811) visited the type locality of *Gymnostomum riparium* ("*in agro vindobonensi in saxis, palis ad Danubii ripas*"; Host 1797) together with Host who showed him plants of the species he described. Hence Hedwig's description, '"*peristomii dentes* […] *ferrugineo-fusci*", is most likely based on these plants since he only mentions the type locality of *Trichostomum nigricans* in Switzerland as a further locality of the species (Schwaegrichen 1811). Bruch *et al.* (1842) described the colour of peristome teeth of *Cinclidotus riparius* as '"lutescenti-ferruginea" and the basal part of the peristome as "croceo-rubellum". They also provide the description of the peristome surface structure as '"tenuissime granulosum". This description and Schkuhr's (1810) picture plate provided further evidence that the peristome of *C. riparius* may have been erroneously described, or that only an extreme of its variability was described by many subsequent authors (see Limpricht 1890; Amann & Meylan 1912; Mönkemeyer 1927; Burck 1947; Pedrotti 2001; Smith 2004).

To clarify the characteristics of the peristome of *C. riparius* and the taxonomic status of *C. confertus*, we studied Euro-

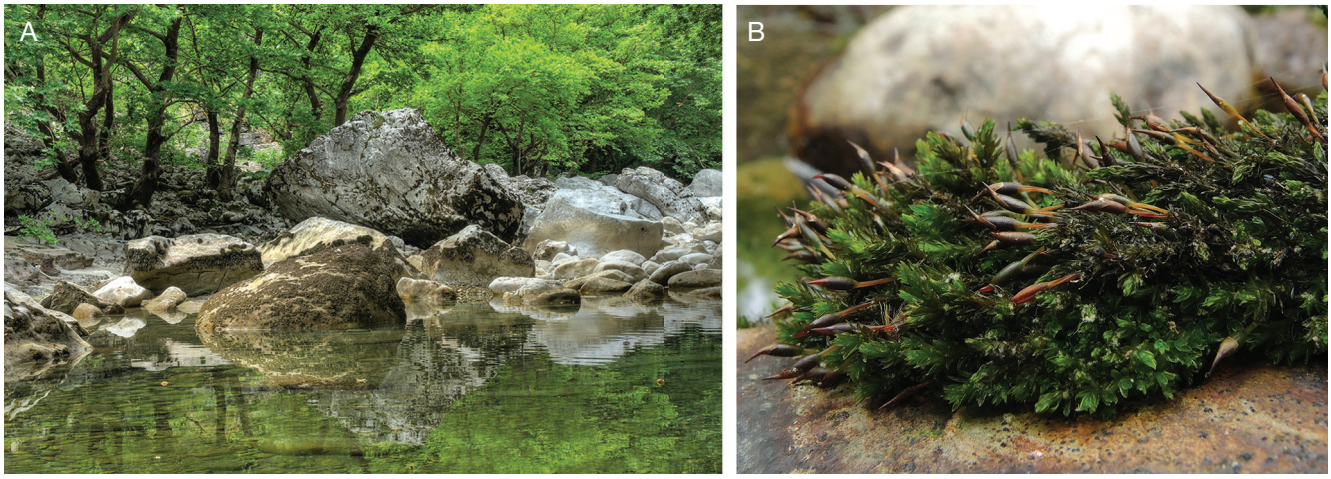

Fig. 1. — *Cinclidotus confertus* Lüth at the type locality in Vikos Gorge (Epirus, Greece): **A**, Bolder where the type specimen was collected in 2000; **B**, Sporophyte bearing cushion of *C. confertus*. Photos: M. Lüth.

pean specimens, including the type material of *C. confertus* and *T. nigricans* morphologically, having particularly examined the variability of the colour and ornamentation of the peristome. We also obtained molecular data from specimens corresponding to both taxa, including material from the type locality of *C. confertus* and further newly discovered localities.

METHODS

The study is based on herbarium material from B, BOZ, CBFS, JE and private collections of the authors including the lectotype of *Trichostomum nigricans* Brid. (herbarium B; Zippel 2006) and an isotype of *C. confertus* (priv. herb. M. Lüth, ML 2805; Appendices 1, 2).

Morphology

To study the morphological variability of *Cinclidotus riparius* we examined specimens from Austria, Czech Republic, France, Germany, Italy, Macedonia, Montenegro and Switzerland (Appendices 1, 2). Specimens of *C. riparius* (including synonyms) in the herbaria mentioned above were screened for the presence of sporophytes. Of these, 24 had sporophytes, and in 20 specimens the peristome was preserved in a state that allowed an evaluation of its characteristics. For *C. confertus* initially only type material was available, which derived from a collection made on a single boulder (Lüth 2002). We thus revisited the type locality to make further collections and to search for further occurrences in the region. At the type locality in the Vikos Gorge (Ioannina, Epirus), we could sample a large population (Fig. 1). Numerous cushions including sporophyte bearing plants were observed in a transect of *c.* 200 m along the Vikos river. Furthermore, we sampled a second population (plants showing the same morphological characteristics) in the Aoos Gorge near Konitsa (Epirus; Appendices 1, 2). In total, we studied 11 specimens assignable to *C. confertus* with well-preserved peristome.

We superficially compared the habit of plants (plant size, branching pattern and density of foliage), and examined the leaf posture in wet and dry state (straight/flexuose, appressed/loosely appressed/erect/patent and combinations of these states), leaf shape (oblong/lanceolate/lingulate/ovate and combinations of these), shape of leaf apex (rounded/shortly mucronate/long mucronate), lamina structure (unistratose/bistratose), peristome colour (yellow/orange/red/brown and combination of these) and peristome ornamentation (smooth/minutely rough/rough/slightly papillose/moderately papillose strongly papillose) and ornamentation of the lamina cells (smooth/slightly papillose). Cell size in the middle and upper part of the lamina, seta length, capsule length and spore size were quantitatively assessed. All characteristics were analysed microscopically using the dissecting and compound microscopes.

Molecular analysis

Data set

The sampling included two specimens of *Cinclidotus confertus*, three specimens of *C. riparius* from central and southern Europe supplemented with sequences from one specimen retrieved from GenBank, one specimen of *C. aquaticus* (Hedw.) Bruch & Schimp., one of *C. fontinaloides* (Hedw.) P.Beauv. and two of *Dialytrichia mucronata* (Brid.) Broth. Sequences from *Aloina rigida* (Hedw.) Limpr. and *Barbula unguiculata* Hedw. were used as outgroup representatives (Appendix 1). One of the *C. confertus* specimens was from the type locality in the Voidomatis River and the other from Aoos river, *c.* 10 km NE of the type locality. These rivers come together *c.* 10 km downstream of the sampling sites. We decided to sample two molecularly informative and well-represented loci in the Pottiaceae, nrITS2 (ribosomal internal transcribed spacer 2 with parts of adjacent 5.8S and 26S rRNA) and the chloroplast *rps4* gene with the adjacent spacer towards the *trnS* gene (e.g. Werner *et al.* 2004; Kučera *et al.* 2018).

Laboratory protocols

The samples were processed in two labs using different protocols for extraction and amplification. For the samples of *C. confertus* from the type locality and of *C. riparius* from Germany and Italy (Appendix 1) total genomic DNA was extracted using the CTAB-Method (Doyle & Doyle 1990). PCR reactions were performed using TAQ polymerase and PCR-Buffer Y (peqlab, VWR International GmbH, Darmstadt, Germany). For a 50 µl reaction volume, 5 µl 10× buffer Y, 1 µl 10 mmol dNTPs solution, 2 µl of each primer (10 pmol/µl), 0.5 µl TAQ polymerase, 1 µl DNA-extract and 38.5 µl water were used. For the *rps4* region the primers rps5 (Nadot *et al.* 1994) and trnas (Buck *et al.* 2000) and for the ITS2 region the primers ITS3 and ITS4 (White *et al.* 1990) were used. The temperature profile of PCR reaction for both regions was 3 minutes denaturation at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 50°C and 1 minute at 72°C, and a final elongation step of 5 minutes at 72°C. Successful amplification was tested visually using ethidium bromide in Gel electrophoresis. PCR fragments were cut out of the gel and purified using the QIAEX II Gel Extraction Kit (QIAGEN GmbH, Hilden, Germany). In remaining samples, total genomic DNA was extracted using the NaOH Method (Werner *et al.* 2002). Crude extracts were diluted ×10 (amplification of *rps4*) or ×100 (amplification of ITS) with 100 mM Tris-HCl (pH 8.3). Polymerase chain reactions (10 µl final volume) were performed with 0.6 µl DNA solution, 5 µl Plain PP MasterMix kit (TOP-BIO Ltd, Czech Republic), 2 µl H2O and 1.2 µl of each primer (2.5 pmol/µl). The whole ITS region (ITS1-5.8SrRNA-ITS2) was amplified using the primers m-18-S (Spagnuolo *et al.* 1999) and m-25-R (Stech & Frahm 1999). *Rps4* region was amplified using the primers rps5 (Nadot *et al.* 1994) and the trnas (Buck *et al.* 2000). The amplification cycle for *rps4* started with 5-minute denaturation at 95°C, followed by 40 cycles of 1 minute at 95°C, 1 minute at 58°C, and 2 minutes at 65°C, and a final extension step of 10 minutes at 6°C. The amplification cycle for ITS started with 3-minute denaturation at 94°C, followed by 40 cycles of 30 seconds at 94°C, 30 seconds at 52°C, and 1 minute at 72°C, and a final extension step of 10 minutes at 72°C. Successful amplifications, visualized using GelRed dye (Biotium Inc., Hayward, CA, United States), were cleaned with a mixture of one unit exonuclease I (20 U/µL; EN0581) and two units alkaline phosphatase (1 U/µL; EF0651, Thermo Fisher Scientific, Waltham, United States). For some samples data obtained from the direct ITS sequencing indicated a mixed template and more than two polymorphic positions within one sequence. In these cases, we applied cloning techniques following the procedure described by Košnar *et al.* (2012) to separate and sequence the different templates. Purified fragments from both labs were commercially sequenced using the amplification primers by GATC Biotech AG (now part of Eurofins Scientific SE, Luxembourg).

Sequence editing, alignment, and phylogenetic analysis

The raw sequences were edited (trimming of primer complements, interpretation of ambiguities where possible) in BioEdit v7.2.6 (Hall 1999) and Geneious v11 (Biomatters, available from http://www.geneious.com/). The nrITS1-locus of the specimens of which the whole ITS region was sequenced was excluded from the alignment and phylogenetic analysis. Sequences were aligned using the online interface of MAFFT v7 (Katoh & Standley 2013). For the *rps4* locus, we employed the E-INS-i strategy and for the ITS2 locus the Q-INS-i strategy and default settings for all other parameters. The E-INS-i strategy is an iterative refinement method combining weighted sum-of-pairs (Gotoh 1995) and COFFEE-like score (Notredame *et al.* 2000), which evaluates the consistency between a multiple alignment and pairwise alignments (Katoh & Standley 2013). The Q-INS-i strategy uses a sequence-based pairwise alignment algorithm together with a consistency function which incorporates secondary structure information of RNA in the iterative refinement step (Katoh & Toh 2008a; b). This latter strategy is suitable for alignment of diverged sequences whereas the advantage for alignment of conserved sequences (such as *rps4*) is small (Katoh & Toh 2008a). For the alignment of the variable ITS2 sequences the Q-INS-i strategy performed better (less non-homologies) than strategies with simpler algorithms implied in Mafft (we tried different strategies; cf., e.g. Xia *et al.* 2016; Kučera *et al.* 2017). The resulting alignments were manually corrected in a few sections where non-homologies were observed.

Phylogenetic analyses were performed using Bayesian inference (BI) and maximum likelihood (ML) methods. For BI we used MrBayes v3.2.6 (Ronquist *et al.* 2012) and for ML we used RAxML v8.2.4 (Stamatakis 2014). Initially, we analysed the two loci separately and for the ITS2 locus we tried two different options, once using DNA data only and once considering also indels. These were coded by applying the simple indel coding method (Simmons & Ochoterena 2000) using SeqState v1.4 (Müller 2005). In the *rps4* alignment, only the sequence of the outgroup species *Aloina rigida* (Appendix 1) showed an insertion at a single position. Therefore, we have not scored indel data for *rps4*.

In the initial (separate) analyses we included all ITS2 variants observed in the samples. The sample of *C. riparius* from Montenegro and one sample of *D. mucronata* had two ITS2 variants each and the samples of *C. aquaticus*, *C. fontinaloides* and the second *D. mucronata* sample had three variants each. Optimal partitioning scheme and best-fit substitution models available in MrBayes and RAxML were identified using the Partitionfinder2 software (Lanfear *et al.* 2016) using the 'greedy' algorithm and the AICc for model selection. For *rps4*, four partitions were evaluated, each codon position and the non-coding section of the locus. For ITS2, the DNA partition was evaluated as a whole. As proposed by the Partitionfinder, in the BI analyses we specified the HKY-Model (Hasegawa *et al.* 1985) for codon position one and two of the *rps4* gene and the GTR model for codon position three, the non-coding section of the locus and the DNA partition of ITS2. For the indels partition of the ITS2 locus (if scored) we specified a binary restriction site model (nst=1). These analyses were run with unlinked parameters for the respective partitions, a sample frequency of 100 and default settings for all other

TABLE 1. — Loci and alignment lengths of the concatenated dataset and partitions and substation models used in the Bayesian inference (**BI**) and Maximum likelihood (**ML**) analyses.

Locus	Length [bp]	Partitions	Length [bp]	Substitution model BI	Substitution model ML
rps4	658	Codon position 1 and 2	392	HKY (Hasegawa *et al.* 1985)	GTR+G
		Codon position 3, spacer	266	GTR	GTR+G
ITS2	567	ITS2	567	GTR+G	GTR+G

parameters. RAxML offers limited substitution model options and only one model ca can be defined for all partitions of an analysis. We chose the GTR+G model for all initial ML analyses. This model virtually includes the different models proposed by Partitionfinder2 for the different partitions of the analyses (see Stamatakis 2016). Support for the nodes of the best scoring tree out of 50 independent ML runs was assessed using the thorough bootstrapping algorithm (Felsenstein 1985) with the extended majority rule bootstopping criterion (Pattengale *et al.* 2010).

The different ITS2 sequences of samples with polymorphic ITS2 sequences were clustered as follows: the variants of the *C. aquaticus* and *C. fontinaloides* samples were resolved as monophyletic for the respective sample with maximum robustness of nodes (1/100). The five variants of the two *D. mucronata* samples were resolved as monophyletic (0.9/83) and, within this clade the three variants of the sample from Great Britain were resolved as monophyletic (0.9/-) and the two variants from the sample from Portugal remained unresolved. The two variants of the *C. riparius* sample from Montenegro were clustered (0.99/87) with the sequences of other *C. riparius* and *C. confertus* samples, with one variant identical to sequences of other *C. riparius* and *C. confertus* samples and the other resolved within the same polytomy. This pattern allowed to select a random variant from the samples with polymorphic ITS2 for the following analyses. The topology of the phylogenetic trees of ITS2 sequences was identical for the two indels options with very similar support values of nodes. Because of the high variability of ITS2 sequences at several positions of the alignment it was unclear for a considerable portion of the scored indels if they represented true homologies and we therefore decided not to consider indel data in the final analysis. Since no contrasting signals from the two loci where found we conducted partitioned analyses on the concatenated dataset of the two loci.

For this final analyses optimal partitioning scheme and best-fit substitution models were identified in the same way and considering the same DNA-partitions as for the initial analyses (Table 1).

The BI analysis was run with unlinked parameters for the respective partitions. For codon positions one and two of the *rps4* gene a HKY model (Hasegawa *et al.* 1985) without rate variation across sites was defined. For codon position three of the *rps4* gene and the spacer towards the *trnS* gene a GTR model without rate variation across sites and for the ITS2 partition a GTR model with a gamma-shaped distribution of rates across sites was used. Two simultaneous runs were performed for 30 m. generations with 16 chains, a temperature of 0.01, a sample frequency of 100 and otherwise default settings. Convergence was evaluated using Tracer v1.6.0 (Rambaut *et al.* 2013) to check that all ESS values exceeded 200. Fifty percent majority rule consensus tree was calculated after discarding the first 25% trees as burn-in. In the ML analysis, support for the nodes was tested as described above. Bayesian inference posteriori probabilities and ML bootstrap values of each node were visualized using TreeGraph 2.14 (Stöver & Müller 2010).

RESULTS

TAXONOMIC ADDITIONS AND CHANGES

Cinclidotus riparius (Host ex Brid.) Arn.

In Mémoires de la Société Linnéenne de Paris 7: 247 (1827). — *Gymnostomum riparium* Host ex Brid., *Journal für die Botanik* 1800 (1): 274 (1801).

Cinclidotus confertus Lüth, *Cryptogamie, Bryologie* 23: 11-16 (2002), **syn. nov.**

MORPHOLOGY

No clear differences between plants assigned to *C. riparius* or *C. confertus* were observed for any of the morphological characters studied. Both species had straight, erect-patent to patent leaves which are loosely appressed to the stem and slightly flexuous when dry (Fig. 2). In both species, the leaves were oblong-lanceolate to lingulate-ovate, blunt to shortly mucronate and leaf shape varied considerably within and between specimens. The lamina was usually unistratose and smooth in both taxa. However, leaves with a few bistratose spots and slightly papillose laminal cells were observed in some specimens of both taxa. In *C. riparius*, the cell pattern in the middle and upper part of the lamina was heterogeneous with cells of different sizes and cell width varying between 6.4 and 16 µm. *Cinclidotus confertus* showed a similar cell pattern with cells (6)10-20 µm wide. Spore diameter in *C. riparius* ranged between 12 and 31 µm and between 14 and 32 µm in *C. confertus*. In both taxa the spores within each sporangium with well-developed spores were somewhat dimorphic with two predominant sizes, spores that are around 15 µm and spores around 25-30 µm in diameter. Seta length varied between 3 and 6 mm in *C. riparius* and between 3.5 and 6 mm in *C. confertus*. Capsule length varied between 1.5 and 3 mm in *C. riparius* and between 1.5 and 3.5 mm in *C. confertus*.

In most specimens assigned to *C. riparius*, the peristome had a distinct reddish tinge with colours ranging from orange to red or reddish-brown. This colouring was retained even in specimens more than 100 years old (Fig. 3). Such specimens mostly showed a reddish-brown peristome. In a few specimens a pale, yellowish peristome was observed. However, this usually coincided with poorly developed

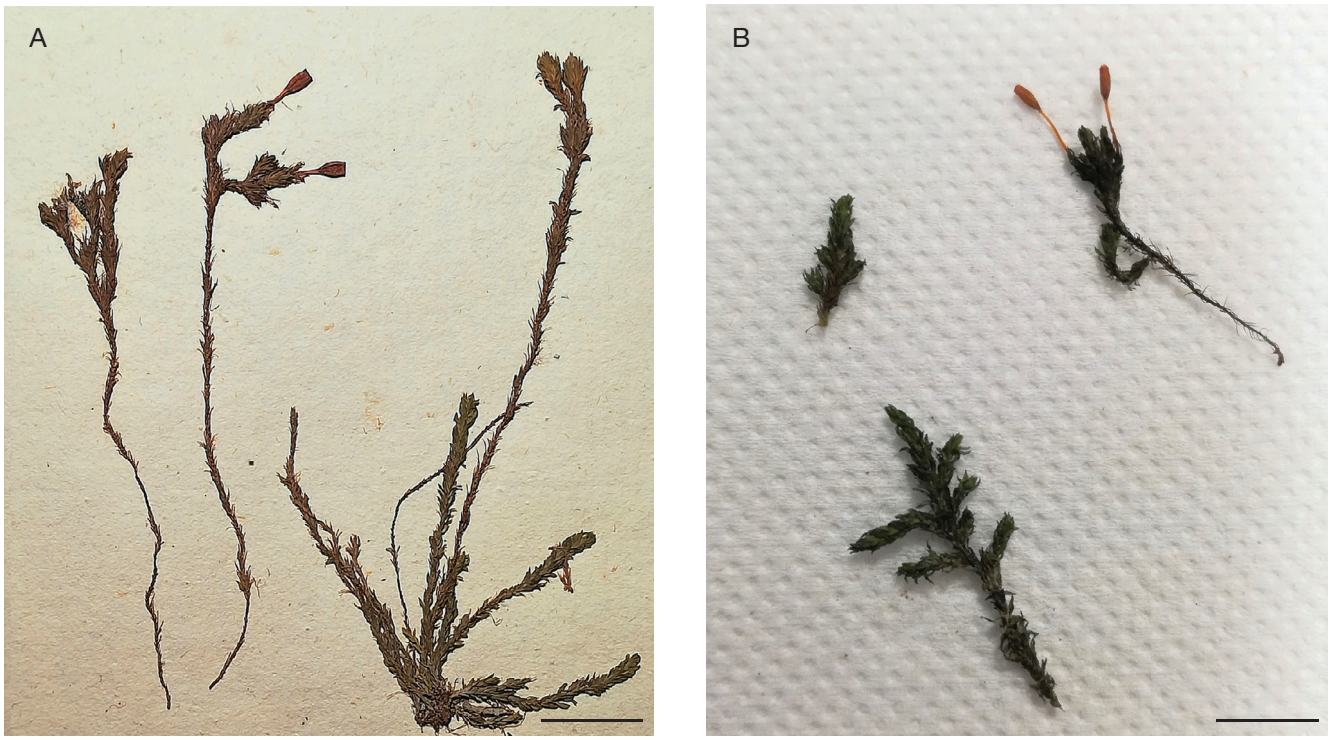

Fig. 2. — Habit of **A**, *Cinclidotus riparius* (Host ex Brid.) Arn. (Lectotype of *Trichostomum nigricans* Brid., B 31 017701-1) and **B**, *C. confertus* Lüth (T.Kiebacher 930). Scale bars: 1 cm. Photos: T. Kiebacher.

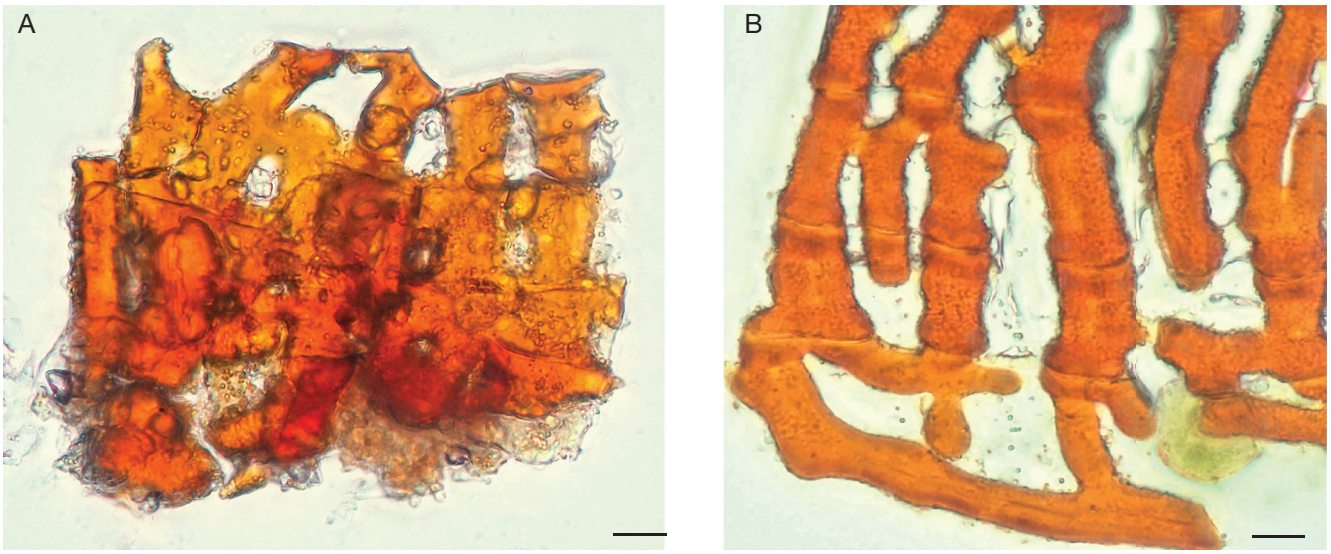

Fig. 3. — Basal part of the peristome of **A**, *Cinclidotus riparius* (Host ex Brid.) Arn. (Lectotype of *Trichostomum nigricans* Brid., B 31 017701-1) and **B**, *C. confertus* Lüth (T. Kiebacher 930). Scales bars: 10 µm. Photos: T. Kiebacher.

capsules or with old capsules where only remnants of the peristome could be found. The lectotype of *Trichostomum nigricans* has two capsules with fragmentary peristome. The peristome teeth are largely broken off. Only a small basal remnant from the capsule mouth could be examined. This remnant was reddish-brown (Fig. 3). In the specimens assigned to *C. confertus* the peristome was consistently red to reddish-brown, rarely orange (Fig. 4). No difference in the ornamentation of the peristome was found between the two taxa. Generally, the papillosity varied considerably between slightly (but distinctly) to strongly papillose teeth surface in both taxa. Minutely rough peristome teeth were only observed in two specimens assigned to *C. riparius*. The peristome remnants from the lectotype of *T. nigricans* were somewhat rough and had irregularly scattered, but distinct papillae (Fig. 3). In specimens from both taxa the extreme base of the peristome (below the capsule mouth) was usually slightly or distinctly paler (yellowish, orange

or light brownish) and less papillose with more scattered and lower papillae.

Molecular analysis

The number of variable sites in the alignment was 247 for ITS2 and 47 for *rps4*. All sequences from *C. riparius* and *C. confertus* appeared in a monophyletic clade supported by maximum posteriori probability and bootstrap values (Fig. 5). Within this clade two samples of *C. riparius* from Montenegro and Northern Italy and the two samples of *C. confertus* were separated in a weakly supported clade. This clustering originated from a single transition in ITS2 shared by these samples. The same transition was also present in the second ITS2 sequence of the *C. riparius* sample from Montenegro which was not used in the final analyses. Furthermore, the ITS2 sequence of *C. riparius* from Germany differed from all other samples of *C. riparius* and *C. confertus* in a single base insertion. All other sites in the ITS2 alignment as well as the whole *rps4* alignment were identical for all samples of *C. riparius* and *C. confertus*.

DISCUSSION

In Europe, *Cinclidotus riparius* has a southern distribution, being widespread in countries bordering the Mediterranean Sea, while it is rare in central and northern European countries (Blockeel 1998; Hodgetts 2015). In central Europe, sporophytes have been rarely observed (Lambinon & Empain 1973; Touw & Rubers 1989; Blockeel 1998; Nebel & Philippi 2000; Smith 2004; pers. obs.) and in Britain and Ireland only female plants are known (Blockeel 1998). Outside Europe, *C. riparius* is known from North Africa and central and Northwest Asia (Frey & Kürschner 1991; Ignatov & Afonina 1992; Ros *et al.* 1999; Kürschner 2008).

In our study, high morphological variability was observed in both gametophyte and sporophyte characteristics of the analysed *Cinclidotus riparius* and *C. confertus* samples. The variability of *C. confertus* is largely within the range of variability of *C. riparius*. Most importantly, the colour and ornamentation of the peristome, which were used as diagnostic characters of *C. confertus*, seem to be identical between the two taxa. Typically, both taxa have a reddish and distinctly papillose peristome. The occasional occurrence of yellowish coloured peristome in *C. riparius* seems to represent the extreme expression of the variability. However, hybrid origin of sporophytes where the male gamete was contributed by another *Cinclidotus* species could also be an explanation for such untypical expressions. It is known from different moss families, including Pottiaceae, that hybrid sporophytes can differ in shape, size and colour, depending on the hybrid parents of the sporophyte (Wettstein 1924; Pettet 1964; Natcheva & Cronberg 2004; Rensing *et al.* 2013). Hybridization is quite common in mosses (Natcheva & Cronberg 2004) and within the genus *Cinclidotus,* hybrid origin was suggested for *C. danubicus* Schiffn. & Baumgartner (Nebel & Philippi 2000; Ahamed & Frahm 2003).

Fig. 4. — Capsule of *C. confertus* Lüth (T. Kiebacher 930) with reddish-brown peristome. Photo: T. Kiebacher.

Marginal morphological differences could only be observed in cell size with a broad overlap between the two taxa. Furthermore, sequences from the *rps4* and ITS2 loci are either identical or very similar (one transition) for specimens assigned to the two taxa. Consequently, we propose to synonymize *C. confertus* with *C. riparius*. The distinction of the two species was based on erroneous or incomplete descriptions of the peristome in the bryological literature (e.g. Smith 2004; Pedrotti 2001). These descriptions of a yellow and smooth or almost smooth peristome may be related to the fact that *C. riparius* in central Europe rarely produces sporophytes (Lambinon & Empain 1973; Touw & Rubers 1989; Blockeel 1998; Nebel & Philippi 2000; Smith 2004; pers. obs.). Hence, authors have followed previous descriptions based on untypical specimens

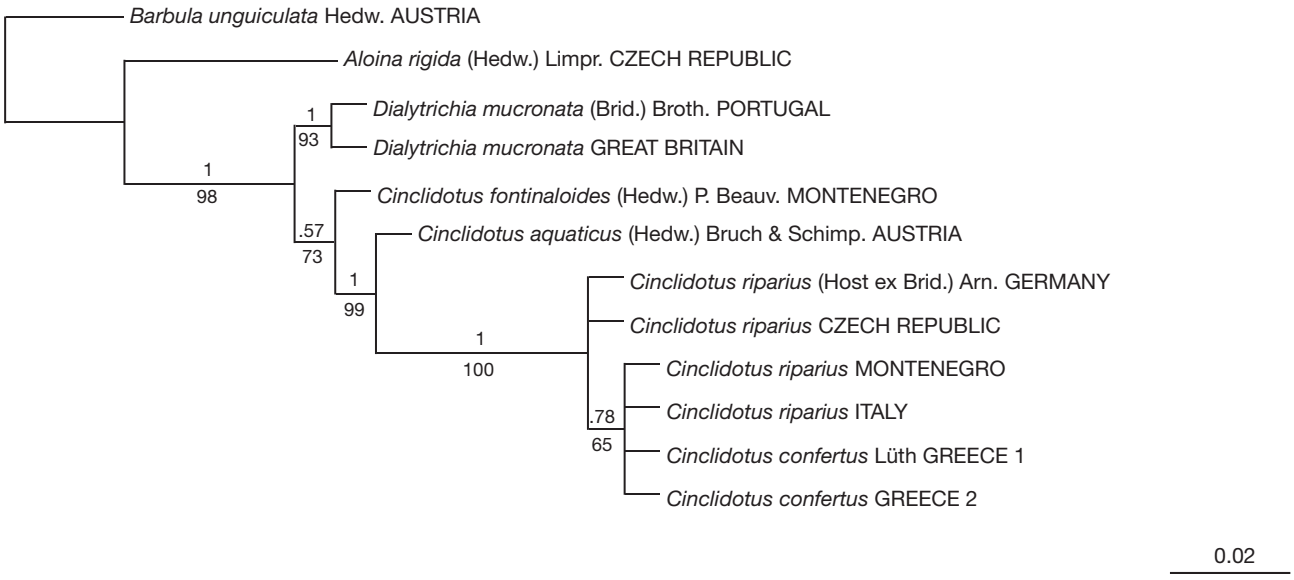

Fig. 5. — Bayesian inference (50% majority rule consensus tree) on the concatenated dataset of *rps4* and ITS2 sequences. Numbers above branches indicate posterior probability from the BI analysis. Numbers below branches indicate the bootstrap support (extended majority rule bootstopping criterion; Pattengale *et al*. 2010) from the ML analysis. GenBank accession numbers of the sequences are given in Appendix 1.

or poorly conserved herbarium material. Furthermore, the peristome is quite fragile and frequently eroded soon after the lid dehiscence (Bruch *et al.* 1842).

The four specimens grouped by a weakly supported branch within the clade of *C. riparius and C. confertus* represent the southern part of the geographical range considered. They all originate from Southern European countries. In contrast, the two samples which appeared separated from this group in the phylogenetic analyses are from Central Europe. This clustering may indicate limited gene flow between the two populations which might depend on the main European watershed. The samples from Germany and Czech Republic are from watersheds which discharge to the North Sea, whereas the other four are all from water courses which discharge to the Mediterranean Sea. Furthermore, dispersal of spores from northern populations is limited because they rarely produce sporophytes (Lambinon & Empain 1973; Touw & Rubers 1989; Blockeel 1998; Nebel & Philippi 2000; Smith 2004; pers. obs.). *Cinclidotus riparius* is dioicous and it seems that with increasing latitude male plants become rare (pers. obs.) and they are unknown in Britain (Smith 2004). Genetic differences between southern and northern populations in Europe, attributed to postglacial recolonization from different refugia, have been observed in a number of other moss species (Shaw *et al.* 2011).

High phenotypic variability (as observed here in *C. riparius*) is a well-known pattern in aquatic bryophyte species (Warnstorf *et al.* 1913; Watson 1919; Lodge 1959; Wehr & Whitton 1986; Hedenäs 2008; Spitale & Petraglia 2010) and has often led to overestimates of the taxonomic value of characters like a bi- or multistratose lamina. This resulted in the description of new species which had to be reduced to synonymy later (e.g., Hedenäs 2008; Spitale & Petraglia 2010). In a number of examples morphological differentiation of aquatic species has been shown to depend on phenotypic plasticity in response to the environment rather than on genetic differentiation (Vanderpoorten & Jacquemart 2004; Buryová & Shaw 2005; Hedenäs 2008). On the other hand, genetic differentiation has been observed within taxonomic units which formerly, based on morphological similarity where considered as a single species. Such cryptic patterns of speciation, including reproductive isolation and polyphyly are known to occur between large geographical units (i.e., continents) as well as sympatrically (Shaw & Allen 2000; Hedenäs & Eldenäs 2007; Hedenäs 2008; Hutsemékers *et al.* 2012).

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Appendix 1. — Specimen data and GenBank accession numbers for the molecular analyses. Accession numbers of newly generated sequences are marked in **bold**. Data format: Nation: Region: Locality, Altitude, Coordinates, Date, Collector (Herbarium acronym registration No.; GenBank Accession numbers for *rps4*, ITS2). **ML**, M. Lüth; **TK**, T. Kiebacher.

Aloina rigida (Hedw.) Limpr.

Specimen. — **Czech Republic**. Břeclav District: Pavlov, *s.d.*, Košnar (CBFS[CBFS 15124]; JX679952, JX679976).

Barbula unguiculata Hedw.

Specimen. — **Austria**. Carinthia: Heiligenblut, *s.d.*, Kučera (CBFS[CBFS 12829]; HM147804, HM147777).

Cinclidotus aquaticus (Hedw.) Bruch & Schimp.

Specimen. — **Austria**. Upper Austria: Pießlingursprung, 760 m a.s.l., 27.IX.2003, Kučera (CBFS[CBFS 11079]; **MK031725**, **MK031717**).

Cinclidotus confertus Lüth

Specimens. — 1: **Greece**. Epirus: Vikos Gorge between Vikos and Monodendri, 495 m a.s.l., 07.VI.2015, ML & TK (*priv. herb.* ML 8158; **MK314718, MK036235**); 2: Epirus: Aoos Gorge between Konitsa an Stomio monastery, 460 m a.s.l., 40.025417°N, 20.768278°E, 09.VI.2015, TK & ML (CBFS[CBFS 20056], *priv. herb.* TK 944; **MK031726**, **MK031718**).

Cinclidotus fontinaloides (Hedw.) P. Beauv.

Specimen. — **Montenegro**. Mojkovac: Tara river near Dobrilovina, 710-720 m, 43.02646°N, 019.40868°E, 30.VII.2007, Košnar (CBFS[CBFS 13270]; **MK031721**, **MK031713**).

Cinclidotus riparius (Host ex Brid.) Arn.

Specimens. — **Czech Republic**. Havlíčkův Brod Distr.: Sokolohrady, *s.d.*, Košnar (CBFS[CBFS 13042]; JX679940, JQ890469). — **Germany**. Baden-Württemberg: Efringen, Isteiner Schwellen, 223 m a.s.l., 47.646611°N, 07.543861°E, 14.IV.2014, TK (*priv. herb.* TK 828; **MK314716**, **MK036233**). — **Italy**. Alto Adige: Brixen, Milland, 0.7 km N Kampan, 550 m a.s.l., 08.II.2006, Hilpold (BOZ[BOZ BRYO 144]; **MK314717**, **MK036234**). — **Montenegro**. Mojkovac: Tara river near Dobrilovina, 710-720 m, 43.02646°N, 019.40868°E, 30.VII.2007, Košnar (CBFS[CBFS 13271]; **MK031722**, **MK031714**).

Dialytrichia mucronata (Brid.) Broth.

Specimens. — **Great Britain**. Derbyshire: Ashbourne, Bentley Brook, 115 m a.s.l., 10.X.2008, Blockeel (CBFS[CBFS 16377]; **MK031724**, **MK031716**). — **Portugal**. Lisboa: Montes Claros, 120 m a.s.l., 38.71600°N, 09.204°W, 07.V.2013, Sérgio (CBFS[CBFS 16064]; **MK031723**, **MK031715**).

Appendix 2. — Selected specimens of *C. riparius* and *C. confertus* studied morphologically in addition to the ones used in the molecular analyse (Appendix 1). Data format: Nation: Region: Locality, Altitude, Coordinates, Date, Collector (Herbarium acronym registration No.; GenBank Accession numbers for *rps4*, ITS2). **ML**, M. Lüth; **TK**, T. Kiebacher.

Cinclidotus confertus Lüth.

Specimens. — **Greece**. Epirus: Vikos Gorge between Vikos and Monodendri, 513 m a.s.l., 39.944639°N, 20.716444°E, 07.VI.2015, TK & ML (*priv. herb.* TK 929); loc. cit., 510 m a.s.l., 39.945500°N, 20.715167°E, 07.VI.2015, TK & ML (*priv. herb.* TK 930); loc. cit., 505 m a.s.l., 39.946056°N, 20.714139°E, 07.VI.2015, TK & ML (*priv. herb.* TK 931); loc. cit., 495 m a.s.l., 07.VI.2015, ML & TK (*priv. herb.* ML 8164); loc. cit., 495 m a.s.l., 07.VI.2015, ML & TK (*priv. herb.* ML 8157); loc. cit., 495 m a.s.l., 07.VI.2015, ML & TK (*priv. herb.* ML 8160); *loc. cit.*, 495 m a.s.l., 07.VI.2015, ML & TK (*priv. herb.* ML 8159); loc. cit., 490 m a.s.l., 19.V.2000, ML (iso-, *priv. herb.* ML 2805); Aoos Gorge between Konitsa and Stomio monastery, 445 m a.s.l., 40.031278°N, 20.754528°E, 09.VI.2015, TK & ML (*priv. herb.* TK 943).

Cinclidotus riparius (Host ex Brid.) Arn.

Specimens. — **Austria**. Steiermark: Prettach nächst Leoben, in der Mur, 29.X.1889, C. Glowacki (JE *s.n.*); Ufer der Mur bei Leoben, ca. 550 m a.s.l., 06.VII.1877, J. Breidler (JE *s.n.*); Ufer der Mur bei St. Michael, 07.VII.1884, J. Breidler (JE *s.n.*); Ensfluss bei Gstatterboden im Gesäuse, 560 m a.s.l., 09.VIII.1903, J. Baumgartner (JE *s.n.*); loc. cit., 560 m a.s.l., 09.VIII.1903, J. Baumgartner (B[B 30 0271707]). — **France**. Gard: La-Roque-Sur-Cèze, cascade du Sautadet, 100 m a.s.l., 04.VII.1988, J.L. De Sloover (B[B 30 0268572]). — **Germany**. Baden-Württemberg: Auf Gneisblöcken im Rheine bei Kleinlaufenburg, 06.IX.1862, A. Geheeb (JE *s.n.*); Waldshut, Gross-Laufenburg, 350 m a.s.l., III.1898, T. Herzog (JE *s.n.*); Bayern: Allgäu, im Ochterachtal unweit Hinterstein, 14.IX.1972, A. v. Hübschmann (B[B 30 0240825]); Berchtesgardener Ache, 490 m a.s.l., 01.VIII.1910, I. Familler (JE *s.n.*); Fichtelgebirge, *s.d.*, H.C. Funck (B[B 30 0050496]) ; Nordrhein-Westfalen: Wesseling, am Anlgeplatze der Dampfer, 44 m a.s.l., 08.X.1929, H. Andres (B[B 30 0227217]). Italy: Alto Adige: Brixen, Milland, 550 m a.s.l., 46.704472°N, 11.654194°E, 25.XII.2015, TK (*priv. herb.* TK 1046); loc. cit., 553 m a.s.l., N46.706825° E11.657133°, 03.IX.2017, TK (*priv. herb.* TK 1590). Macedonien: Üsküb: in der Treska, 01.IV.1917, J. Bornmüller (B[B 30 0050535]). Switzerland: no locality provided, *s.d.*, J.C. Schleicher (lectotype of *Trichostomum nigricans*, B[B 31 017701-1]); no locality provided, *s.d.*, Müller (JE *s.n.*). Aargau: Rhein bei Laufenburg, 05.II.1905, A. Geheeb (JE *s.n.*); Neuchâtel: Gorge de l'Areuse, ca. 550 m a.s.l., Mar. 1899, T. Herzog (JE *s.n.*). Schaffhausen: am Rhein bei Laufenberg, 05.II.1905, A. Geheeb (B[B: 30 0050495]); St.Gallen: Toggenburg, Wasserfall Giessenbach, 28.VIII.1905, A. Geheeb (JE *s.n.*).